European Journal of Medical Genetics 54 (2011) 9–13

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Original article

De novo microduplication at 22q11.21 in a patient with VACTERL association

Charlotte Schramm [a,b], Markus Draaken [a,b], Enrika Bartels [a], Thomas M. Boemers [c], Stefan Aretz [a], Felix F. Brockschmidt [a,b], Markus M. Nöthen [a,b], Michael Ludwig [d], Heiko Reutter [a,e,*]

[a] Institute of Human Genetics, University of Bonn, Bonn, Germany
[b] Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany
[c] Department of Pediatric Surgery and Pediatric Urology, Children's Hospital, Cologne, Germany
[d] Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, Germany
[e] Department of Neonatology, Children's Hospital, University of Bonn, Bonn, Germany

ARTICLE INFO

Article history:
Received 26 February 2010
Accepted 6 September 2010
Available online 16 September 2010

Keywords:
VACTERL association
22q11.21
Microduplication
MLPA
SNP array
Cat eye syndrome
Supernumerary der(22)

ABSTRACT

The non-random association of vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with esophageal atresia (TE), renal malformations (R), and limb defects (L) is termed VACTERL association. The aim of the present study was to identify microaberrations characterized by a loss or gain of genomic material that contribute to VACTERL association at a genome-wide level. Molecular karyotyping was performed in a cohort of 12 patients with anorectal malformations and at least two additional cardinal features of the VACTERL association. A *de novo* microduplication at chromosomal region 22q11.21 was identified in a patient presenting with three cardinal VACTERL features (V, A, R) and vesicoureteral reflux, penile hypospadias, caudal regression syndrome, and right-sided congenital equinovarus deformity.

Chromosomal region 22q11.2 is known for its susceptibility to rearrangements. Associated syndromes include the velo-cardio-facial and DiGeorge deletion syndromes, and the complementary 22q11.2 duplication syndrome. The findings of the present study extend the phenotypic spectrum of the 22q11.2 duplication syndrome, and indicate that it also predisposes to VACTERL association. We discuss the overlap between the phenotypic features of our patient and those reported for other 22q11.2 aberrations, and propose that dosage-sensitive loci for all of these phenotypic features may reside on 22q11.2.

1. Introduction

The non-random association of vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with esophageal atresia (TE), renal malformations (R), and limb defects (L) has been termed VACTERL association (MIM #192350) [14,19]. Patients are classified as having VACTERL association when they display at least three of these cardinal features [5]. VACTERL occurs in approximately 1 in 7000 to 1 in 10,000 live births [5]. Nearly all reported cases have been sporadic. However, Solomon et al. [24] recently reported an increased prevalence of isolated VACTERL clinical features in first-degree relatives. Chromosomal abnormalities have been described in rare individual cases and proposed as possible causal factors [23]. These include: (i) deletions of distal 13q [25], ring chromosome 12 [6], and 6q [17]; (ii) duplication on 9q [1]; (iii) mutations in *PTEN* [22], *HOXD13* [12], and *ZIC3* [26]; and (iv) a mitochondrial c.3243A > G substitution [8].

The aim of the present study was to identify *de novo* microaberrations characterized by loss or gain of genomic material (i.e. copy number variants [CNVs]), which may contribute to VACTERL association at a genome-wide level. Molecular karyotyping with 657,366 single nucleotide polymorphisms (SNPs) was performed in 12 case-parent VACTERL trios. A *de novo* microduplication involving chromosomal region 22q11.21 was identified in one patient. These findings extend the phenotypic spectrum of the 22q11.2 duplication syndrome (MIM #608363), and indicate that a gene dosage effect involving the 22q11.2 region predisposes to the manifestation of VACTERL association.

2. Materials and methods

2.1. Subjects

The study was approved by the local Ethics Committee and all families provided written informed consent. Families with

* Corresponding author. Institute of Human Genetics and Department of Neonatology, University of Bonn, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany. Tel.: +49 228 287 22601; fax: +49 228 287 22380.
E-mail address: reutter@uni-bonn.de (H. Reutter).

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doi:10.1016/j.ejmg.2010.09.001

supposed VACTERL association were contacted and recruited through the German self-help organization for patients with anorectal malformations (SoMA e.V.) and the Department of Pediatric Surgery and Pediatric Urology at the Children's Hospital in Cologne, Germany. A total of 26 case-parent trios agreed to participate. Of these, 12 met the study inclusion criterion i.e. the presence of sporadic VACTERL association in the affected patient. As a consequence of the nature of the recruitment procedure, all of these affected individuals displayed anorectal malformations. The clinical features of these patients are summarized in Table 1. All families were of European descent. There was no history of any cardinal features of VACTERL association in the first-degree relatives of the 12 affected patients. Blood samples were taken from all 12 patients and their parents for DNA extraction and the performance of the molecular genetic analyses. In patients with limb defects, Fanconi anemia was excluded prior to blood sampling.

2.2. Array genomic hybridization analysis

Molecular karyotyping was performed using the Illumina Human660W-Quad-v1 BeadChip (San Diego, California), which contains 657,366 SNPs. All analyses were performed in accordance with the manufacturer's protocol. A DNA sample was considered to have failed if less than 95% of the loci were generated on the respective BeadChip. To identify potential CNVs, the SNP fluorescence intensity data of each individual were analyzed with QuantiSNP (v2.1, www.well.ox.ac.uk/QuantiSNP/) using an Objective-Bayes Hidden-Markov model for calling putative CNVs [7]. CNVs with a log Bayes factor of below 30 were discarded, as recommended by Colella et al. (2007) [7]. The results were also checked visually using the GenomeStudio (v2009.2, www.illumina.com/) genotyping module. Here, log *R* ratio values represent a measure of the magnitude of the combined fluorescent intensity signals, and the B allele frequency values denote the relative ratio of the fluorescent signals of the two allelic probes. A duplication is identified by an increase in log *R* and the occurrence of four clusters in B allele frequency (at 0, w0.33, w0.67, and 1). Correspondingly, a deletion is characterized by a decrease in log *R* and a lack of heterozygous state (at 0.5) in B allele frequency.

2.3. CNV analysis

The Cartagenia Bench™ software (Cartagenia n.v., Leuven, Belgium) was used to search the detected CNVs for *de novo* events. This is a database for the management and interpretation of CNV data in routine diagnostics and research. The data sets were analyzed to detect aberrations that were present in the patient but not in the phenotypically normal parents. To be filtered out, the minimum required overlap between a CNV in a patient and that in the parents was set at 80%. Sex chromosome aberrations were excluded from further analysis. The remaining aberrations were checked for gene content (according to UCSC human genome browser assembly build 18 [hg18]).

Results obtained from filtering in the Cartagenia Bench™ database were checked manually. CNVs in patients that were only one or two markers longer than those in parents were excluded. Likewise we excluded CNVs that contained no exonic sequences. We then checked for the presence of these remaining aberrations in our internal database. This includes the data sets of 280 anonymized individuals with diagnoses unrelated to VACTERL association who were genotyped on the Illumina Human660W-Quad-v1 BeadChip. We have established this database to help discriminate between rare polymorphisms and disease-associated variants.

2.4. Quantitative polymerase chain reaction (qPCR)

qPCR was performed as described previously [9]. Primer sequences are available upon request.

2.5. Multiplex ligation-dependent probe amplification (MLPA) analysis

MLPA analysis was performed using the SALSA MLPA kit P250-A1 DiGeorge (MRC-Holland, Amsterdam, The Netherlands) in accordance with the manufacturer's recommendations. This kit contains 48 MLPA probes which cover region 15.959.700–23.283.730 bp on chromosome 22. Amplification products were separated by electrophoresis in a capillary sequencer (AB 3130xl Genetic Analyzer), and raw data were analyzed by Gene Mapper (v4.0, www3.appliedbiosystems.com/). Coffalyser (v9.4, old.mlpa.com/coffalyser/) was used to calculate the relative amount of each amplicon compared to a control DNA sample. Values below 0.78 indicate deletions, and signals exceeding 1.22 indicate duplications [13].

2.6. Paternity testing

Paternity testing was performed using the GenomeStudio (v2009.2, www.illumina.com/) genotyping module in accordance with the manufacturer's recommendations.

3. Results

Molecular karyotyping identified five possible *de novo* aberrations in four of the 12 patients. Four of these were not confirmed by qPCR (data not shown). The presence of a *de novo* microduplication at 22q11.21 was confirmed in one male patient (Case 1; Table 1). This had an estimated size of 2.51 Mb–2.54 Mb (Fig. 1). The first and last duplicated markers were rs450046 at genomic position 17,281,004, and rs140392 at genomic position 19,792,353, respectively (hg18). The flanking markers were rs3810600 at genomic position 17,273,248, and rs28435917 at genomic position 19,813,782. The breakpoint regions can therefore be defined as ranging from: (i) positions 17,273,249 to 17,281,003, and (ii) positions 19,792,354 to 19,813,781. This finding was confirmed by MLPA analysis, which identified duplications for probes CLTCL1 to LZTR1 (data not shown).

The GenomeStudio Reproducibility and Heritability Report revealed a parent–parent–child heritability of 99.98%.

3.1. Case 1

The male patient was the second child of healthy non-consanguineous parents. He had a healthy older brother and the family history was unremarkable. The mother was 28 years of age and gravida 3, para 1 at the time of delivery. The periconception and first trimester periods were uneventful, with no history of maternal infection or exposure to teratogens. At 23 weeks gestation, idiopathic oligohydramnios was detected. This was treated at 24 weeks of gestation with a series of amnioinfusions. Subsequent premature contractions were treated with fenoterol. Following spontaneous delivery at 37 weeks, the patient was noted to have a range of congenital defects. These included high anal atresia with rectoprostatic fistula, caudal regression syndrome comprising dysplasia of the os sacrum and dysgenesis of the os coccygis, right-sided duplicate kidney with vesicoureteral reflux (VUR), right-sided equinovarus deformity and penile hypospadias. Echocardiography and ultrasound of the brain were normal. The anal atresia and penile hypospadias were surgically corrected during the neonatal period. At this time, fusion of the 4th and 5th lumbar vertebrae was